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Cellular Analysis of Circadian Rhythmicity in Cultured SCN Neutrons

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6. AUTHOR(S)
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13. ABSTRACT *(Maximum 200 words)*

Within the mammalian hypothalamus, the suprachiasmatic nucleus (SCN) contains a circadian clock for timing of diverse neuronal, endocrine, and behavioral rhythms. We have tested the hypothesis that the circadian period in behavior expressed by the whole animal is determined by the collective period that arises from the coupling of a large population of clock cells with diverse circadian periods. The results clearly show that circadian period in the whole animal is determined by averaging widely dispersed periods of individual clock cells. We have also shown the inhibitory transmitter GABA can phase-shift individual clock cells in culture. A phase-response curve to GABA has been generated for individual clock cells, and we have shown that daily GABA pulses can synchronize clock cells.

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FINAL REPORT

PI: Steven M. Reppert

Institution: Massachusetts General Hosp
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Grant No: F49620-97-1-0004

Final Report: 2/1/97 - 6/30/99

OBJECTIVES

No change

STATUS OF EFFORT:

Within the mammalian hypothalamus, the suprachiasmatic nucleus (SCN) contains a circadian clock for timing of diverse neuronal, endocrine, and behavioral rhythms. We have tested the hypothesis that the circadian period in behavior expressed by the whole animal is determined by the collective period that arises from the coupling of a large population of clock cells with diverse circadian periods. The results clearly show that circadian period in the whole animal is determined by averaging widely dispersed periods of individual clock cells. We have also shown the inhibitory transmitter GABA can phase-shift individual clock cells in culture. A phase-response curve to GABA has been generated for individual clock cells, and we have shown that daily GABA pulses can synchronize clock cells.

ACCOMPLISHMENTS:

We have fulfilled the goals of Aim 1: to define the intracellular nature of circadian period determination and made significant inroads into Aim 2: to examine the ability of neurotransmitters and modulators to phase-shift clock cells. To test Aim 1, we cultured SCN neurons from wild-type, and heterozygous and homozygous *tau* mutant Syrian hamsters. Our recordings showed that for each genotype, hamster clock cells in the same culture oscillate in different phases and with a wide range of period lengths, similar to what has been previously reported in rats. The large variation among clock cell periods for each genotype appeared to be intrinsic to clock cells because it was similar among the three genotypes.

Despite the wide range of circadian periods, mean clock cell periods in culture was distinct for each genotype demonstrating that the period abnormality of the *tau* mutation is manifested at the single-cell level. For each genotype, clock cell periods show 5- to 15-times more variance compared to the respective variance in circadian periods of wheel-running behavior. The results show that circadian period in the whole animal is determined by averaging widely dispersed periods of individual clock cells.

In addressing Aim 2, we have focused on the inhibitory transmitter GABA. Virtually all SCN neurons are GABAergic and respond to GABA. Moreover, GABA analogs phase shift the circadian clock both in vivo and in vitro. GABA application caused acute inhibition in firing rate of individual clock cells, independent of circadian time. GABA application also phase-shifted SCN clock cells in a time-dependent manner - these effects are mediated through the GABA A receptors. These data demonstrate that single SCN clock cells elicit phase-dependent circadian responses to transmitter stimuli. We have also shown that we can synchronize SCN clock cells in culture with GABA pulses. We thus propose that GABA is an important synchronizer of SCN neurons in vivo.

PERSONNEL SUPPORTED:

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David R. Weaver, Associate Professor of Pediatrics, Children's Service, Massachusetts General Hosp., and Harvard Medical School

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PUBLICATIONS:

Liu C, Weaver DR, Strogatz S, Reppert SM. Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei. Cell 1997; 91, 855-860.

Reppert SM. A clockwork explosion! Neuron 1998; 21, 1-4.

Liu C, Reppert SM. GABA sychronizes clock cells within the suprachiasmatic circadian clock. Neuron in press.

INTERACTIONS/TRANSITIONS:

A. Meetings:

Oral presentation, Society for Research on Biological Rhythms, Florida, May 1998.

Oral presentation, FASEB Meeting on Entrainment, Snowmass, CO, July, 1998

Oral presentation, Chronobiology Gordon Conference, Barga, Italy, June, 1999.

B. Consultative

None

C. Transitions:

None

NEW DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES

None

HONORS/AWARDS

During grant period: None

Lifetime for SM Reppert:

1987-	American Society for Clinical Investigation
1989	E Mead Johnson Award for Outstanding Research in Pediatrics
1992-	NIH-NICHD MERIT Award
1996-	Editorial Board, Neuron